Counting Spores in a Cluster

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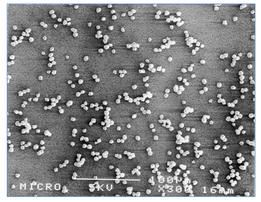
Introduction

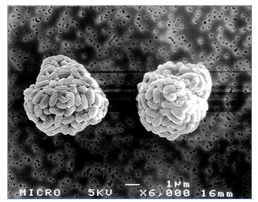
Aerosolized spores are often encountered as aggregates in nature and in the laboratory, and the question of how many spores comprise a particle of a given size arises. We will show a direct empirical method for determining the relationship between cluster size and spore count that does not rely on assumptions about spore size, packing fractions, culturable fraction, etc., providing greater accuracy than currently employed methods.

From a randomly distributed dispersion of spores in water, we produce a sequence of small droplets of uniform size using drop-on-demand technology. The number of spores per droplet will follow a Poisson distribution. Some of these droplets are made to impact along a row on a microscope slide from which they are observed under a microscope and the spores, which lay flat in the splat circles, are counted, typically for a few hundred droplets. Other droplets are passed through an oven to dry down to clusters which are then sized with a TSI Aerodynamic Particle Sizer (APS). The number of spores in a cluster should be proportional to the cluster's diameter cubed (d^3); the proportionality constant is found by associating the most probable spore count from the splats with the most probable APS (volume) diameter. Repeating the measurements at a few other concentrations (particle sizes) yields the same proportionality constant and verifies the assumed d^3 dependence.

Materials & Methods

The Scanning electron microscopy (SEM) photo below (Figure 1) shows a nearly monodisperse field of particles, 5 to 6 µm in diameter, made of aggregated Bacillus atrophaeus (Bg) spores. These were formed by making 100 pl droplets from a Bg-in-water suspension using an Ink Jet Aerosol Generator (IJAG) and drying the droplets in warm air to leave residue particles of Bg clusters. Figure 2 is a close up SEM image of a pair of the clusters. Our question is: how many individual Bg spores are in the clusters? More generally, what is the relationship between a cluster's size and the number of cells comprising it?





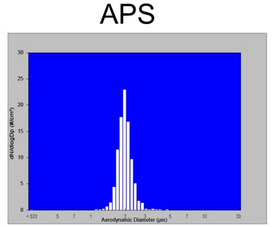
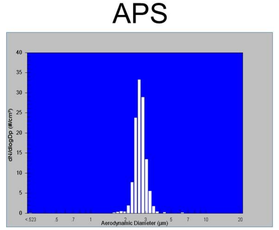
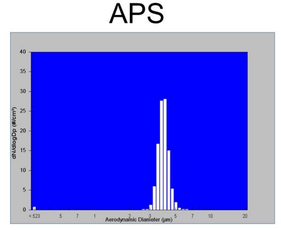
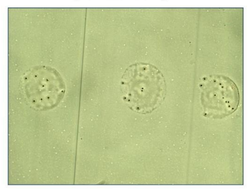
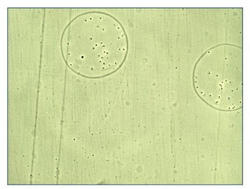
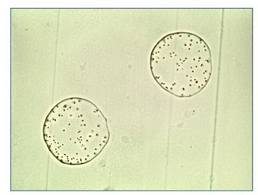
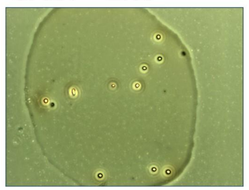
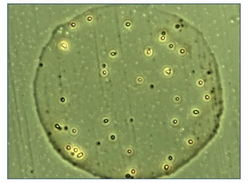
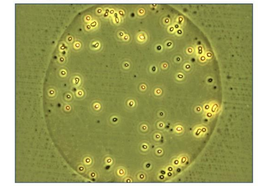
A slurry of cleaned Bg spores in clean water is prepared; the greater the spore concentration the larger the size of the final cluster. The IJAG produces a series of uniformly sized droplets which are dried down to clusters and presented to the inlet of an APS for sizing. Another series of droplets is made in the same fashion with the same slurry, but are not dried. Instead, the wet droplets are allowed to impact upon a moving clean glass slide, resulting in a line of circular splats that quickly dry leaving individual spores in a layer distributed over the splat area. The spores can be seen and counted under an optical microscope.

Even if the droplets were precisely uniform in size, the spores are distributed randomly in the slurry and so the number of spores per droplet (i.e., per splat) follows a Poisson distribution about the mean, as does the dried cluster volume. We associate the mean cluster size with the mean spores per splat to answer how many spores per cluster.

Results

We assume the number of spores Ns in a cluster is proportional to the cluster volume, which in turn is proportional to the cube of the aerodynamic diameter da. Thus, $Ns = k\ da^3$, and the task is to measure data allowing the calculation of k, which should depend only on the size of the clustering units, spores in this case. We will calculate k from sets of measurements of three cluster sizes; the constancy of k should validate the simple relation $Ns = k\ da^3$.

To aid counting spores in splats, a small bit of fluorescein, sodium salt, was added only to the splat-forming suspension. This helps reveal the edge of the splat and the spores take on a yellow color. Additionally, the glass slide was wetted and rubbed dry with a solution of tween-80, leaving a very thin layer on the glass to help the splat spread out rather than beading.

APS	APS	APS
		
$N_{particles}$= 984 geo mean = 1.99 µm geo st dev = 1.15	$N_{particles}$= 1231 geo mean = 2.69 µm geo st dev = 1.12	$N_{particles}$= 1070 geo mean = 3.93 µm geo st dev = 1.11
SPLATS	SPLATS	SPLATS
		
N_{splats} = 140 mean = 10 spores/splat st dev = 2.69	N_{splats} = 93 mean =23.6 spores/splat st dev = 4.90	N_{splats} = 69 mean =76.7 spores/splat st dev = 8.14
		
k = 1.27	k = 1.21	k = 1.26

Conclusion

Averaging the three values for k we find k = 1.25 within better than +/-3%. Thus a 2.5 µm cluster contains, on average, about 20 spores, a 5 µm cluster about 156 spores, and so on. This assumes of course that the spores are clean. If the suspension from which clusters are formed contains other materials, suspended or dissolved, that contribute to the volume of the residue particle then there will be correspondingly fewer spores contained.

The technique described here can be applied to any bacteria, spore or vegetative, that can be dispersed singly in a water suspension and is identifiably visible under an optical microscope. For example, we have found that for dry clusters of Staphylococcus epidermidis, k is 0.64 cells/µm^3; and for the very large Bacillus megaterium (vegetative) k is 0.045 cells/µm^3.